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Solid-State ^{13}C Nuclear Magnetic Resonance Spectroscopy of Simultaneously Metabolized Acetate and Phenol in a Soil *Pseudomonas* sp.

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We investigated concentration-dependent primary and secondary substrate relationships in the simultaneous metabolism of the ubiquitous pollutant phenol and the naturally occurring substrate acetate by a *Pseudomonas* sp. soil isolate capable of utilizing either substance as a sole source of carbon and energy. In addition to conventional analytical techniques, solid-state ^{13}C nuclear magnetic resonance spectroscopy was used to follow the cellular distribution of [1-^{13}C]acetate in the presence of unlabeled phenol. With 5 mM acetate as the primary substrate, *Pseudomonas* sp. 9S8D2 removed 1 mM phenol (secondary substrate) at a rate of 2 nmol/mg of total cell protein. Although extensive acetate metabolism was indicated by a significant redistribution of the carboxyl label, this redistribution was not affected by the presence of phenol as a secondary substrate. When the primary and secondary substrate roles were reversed, however, the presence of 1 mM phenol altered the metabolism of 0.1 mM acetate, as evidenced by both the two- to fourfold increases in carboxyl label that appeared in terminal methyl and acyl chain methylene carbon resonances and the decrease in label that occurred in the carbohydrate spectral region. These results suggest that, when phenol is present as the primary substrate, acetate is preferentially shuttled into fatty acyl chain synthesis, whereas phenol carbon is funnelled into the tricarboxylic acid cycle. Thus, simultaneous use of a xenobiotic compound and a natural substrate apparently does occur, and the relative concentrations of the two substrates do influence the rate and manner in which the compounds are utilized. These results also demonstrate the unique advantage of using solid-state nuclear magnetic resonance techniques combined with ^{13}C labeling of specific sites in substrates when doing microbial degradation studies. In this work, the entire cellular biomass was examined directly without extensive extraction, fractionation, or isolation of subcellular units; thus, there is no uncertainty about chemical alteration of substrate metabolites as a result of these often harsh treatments.

Understanding microbial degradation of xenobiotic compounds under conditions similar to those of the natural habitat of the organism is crucial to obtain an accurate assessment of the impact and fate of these chemicals when they are released into the environment. Many factors are involved in the in situ biodegradation of organic compounds, one of the most important being the influence of naturally occurring carbon substrates on rates of degradation and on metabolic pathways used. The effect of various substrates on the microbial degradation of organic pollutants has been the subject of a number of studies (3, 15, 23, 26, 30, 34, 36, 37), many of which used pseudomonads. Pseudomonads have demonstrated the ability to degrade a wide variety of aromatic compounds (6, 20, 21, 23, 27, 35, 38). However, utilization of these compounds by members of the genus *Pseudomonas* apparently can be altered by the presence of more easily metabolized compounds, such as acetate (23, 34), glucose (26, 30), pyruvate (27), arginine (30), and unidentified dissolved organic carbon (34).

The influence of these naturally occurring substrates on the degradation of xenobiotic compounds can be explored by primary-secondary substrate methods (23), in which the compound present at higher concentrations is termed the primary substrate and the compound present at lower concentrations is termed the secondary substrate. We used this approach to investigate concentration-dependent substrate relationships between the ubiquitous pollutant phenol and the naturally occurring substrate acetate (25) during the assimilation and degradation activity of an isolated soil *Pseudomonas* sp. capable of using either substrate as a sole source of carbon and energy. In addition, we used solid-state ^{13}C nuclear magnetic resonance (NMR) techniques, specifically cross polarization (CP) and magic angle spinning (MAS), to investigate the cellular distribution of [1-^{13}C]acetate when it is utilized as either a primary or secondary substrate in the presence of unlabeled phenol. Microbial alteration also plays an important role in the early diagenesis of organic carbon in marine surface sediments, and we recently demonstrated the utility of the ^{13}C CP-MAS NMR technique in distinguishing subtle changes in sedimentary organic carbon with change in depth (8, 9).

MATERIALS AND METHODS

Bacterial isolation and characterization. Subsurface sediments were collected from a creek flood plain margin near Lula, Okla., and subsurface bacteria were isolated as previously described (2).

The isolated pseudomonad, strain 9S8D2, has been tentatively classified as a *Pseudomonas* sp. on the basis of its ability to grow aerobically on glucose, malate, citrate, and acetate but not on sucrose. It is a motile, oxidase-positive, short gram-negative rod capable of producing copious amounts of slime in enriched environments (23). This aerobic rod does not require growth factors.

Culture conditions. Microorganisms were grown in a mineral salts medium (MSM) which contained the following (in

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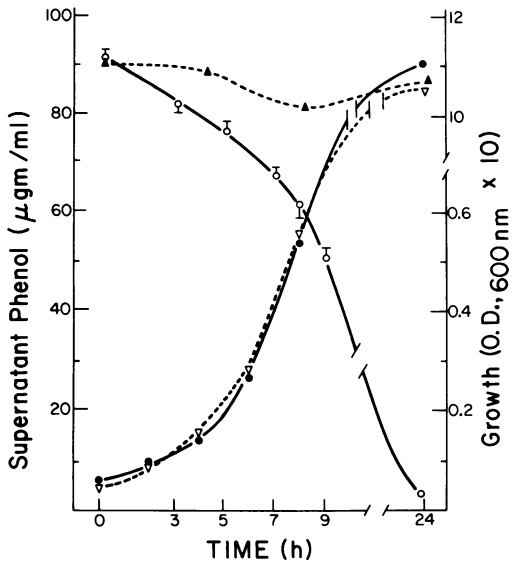

FIG. 1. Growth and uptake of phenol by strain 9S8D2. Cells were added to medium containing 5 mM acetate and 1 mM phenol and incubated for the indicated times at 23°C with agitation. Uptake of phenol was assessed by measuring the amount of phenol remaining in the culture supernatant after rapid centrifugation of culture aliquots. Initial binding or adsorption of phenol to the cells was 6.4 ± 1.9%. Supernatant phenol concentration was measured by gas chromatography for cultures with cells (○) and controls without cells (▲). Culture growth was measured at an optical density of 600 nm for cells in the presence (●) or absence (▽) of phenol.

grams): KCl, 1; $MgCl_2$, 0.2; K_2HPO_4, 7; KH_2PO_4, 0.6; $(NH_4)_2SO_4$, 1; $CaCl_2$, 0.01; and NH_4NO_3, 0.5, per liter of distilled water (final pH, 7.2) and amended with indicated amounts of sodium acetate (NaOAc) or phenol as carbon and energy sources. Stock cultures were maintained on MSM-NaOAc slants at 4°C.

Inocula were prepared by aerobically growing the organisms in MSM with 0.1% NaOAc or 0.1% phenol with agitation at 22 to 25°C, harvesting by aseptic centrifugation at 10,000 rpm (12,000 × *g*) for 10 min (Sorvall RC-5B), washing cells in sterile MSM, and suspending them to the desired density in MSM. Optical density was measured at 600 nm with a Spectronic 20 (Bausch and Lomb).

Experiments were done in final culture volumes of 100 ml to which 5 ml of resuspended culture was added. All incubations were carried out aerobically, with agitation, at 22 to 25°C in darkness. Uninoculated controls were included for all substrates, and cultures were monitored for purity and viability.

In those experiments involving [1-^{13}C]NaOAc, the 99.8% labeled material directly replaced its natural-abundance counterpart in MSM. After microbial growth, monitored by optical density (at 600 nm), cells were harvested as described above, washed in MSM, suspended in a small amount of distilled water, frozen in acetone-dry ice, and lyophilized.

Growth estimations. Growth was monitored by measuring optical density or total cell pellet protein. Protein was assayed by the method of Lowry et al. (24) at 750 nm with a Perkin-Elmer Lambda 3A UV/VIS spectrometer using bovine serum albumin as standard. Optical density was measured at 600 nm with a Bausch and Lomb Spectronic 20 spectrophotometer.

Phenol metabolism. Removal was assayed by extraction of residual phenol from the culture medium. Bacteria were pelleted by centrifugation at 4°C in a Microfuge (model 5412; Beckman Instruments, Inc., Fullerton, Calif.), and 1-ml supernatant aliquots were removed, acidified with 50 μl of 2 N HCl, and extracted into 1 ml of chloroform containing 50 μg of toluene per ml as internal standard. Extracted phenol was analyzed with a Varian 3700 chromatograph equipped with both a glass column (3 m by 2 mm diameter), packed with 60/80 mesh Carbopack B coated with 1% SP-1000, and a flame ionization detector. Phenol peaks were quantitated with a recorder-integrator (model 3390A; Hewlett-Packard Co., Palo Alto, Calif.) using the internal standard (toluene) method. Extraction efficiencies of phenol external standards averaged 80%.

NMR spectroscopy. ^{13}C CP-MAS NMR spectra were obtained with a Bruker WP-200 SY spectrometer equipped with an IBM solids control accessory operating at 50 MHz for carbon. The spectrometer was tuned with *t*-butylbenzene, and chemical shift scales were reported as parts per million downfield from tetramethylsilane. Lyophilized samples were spun at the magic angle at a rate of 4 kHz. A cross polarization program (33) with a 1-ms contact time and a 2-s recycle time was used. Data were collected in 2 kbytes of memory, exponentially multiplied with 20-Hz line broadening, and expanded to 8 kbytes before Fourier transformation (9).

Chemicals. [1-^{13}C]NaOAc, 99.8% atom excess, was purchased from Merck Sharpe & Dohme (Rahway, N.J.) and used without further purification. Organic solvents used for phenol extractions were purchased from Burdick and Jackson Laboratories, Inc. (Muskegon, Mich.) and were of pesticide-residue-grade quality. Phenol was purchased from Aldrich Chemical Co., Inc. (Milwaukee, Wis.). All other chemicals were of reagent quality.

RESULTS

Characterization of primary and secondary phenol metabolism. Utilization of phenol and substituted phenols as sole carbon sources or in the presence of other substrates has been reported previously for *Pseudomonas* sp. (21, 27, 38). Strain 9S8D2 is capable of growth on MSM with acetate as the sole carbon and energy source and requires no additional growth factors. To test the ability of *Pseudomonas* sp. 9S8D2 to grow in the presence of phenol, MSM was amended with 5 mM acetate as primary carbon and energy source and 1 mM phenol as a secondary substrate. Microbial growth and phenol removal from the culture medium over a 24-h period are depicted in Fig. 1. The presence of phenol did not alter growth as assessed by culture optical density or total cellular protein (data not shown). Significant phenol uptake occurred at 5 h of incubation, indicating that, with a short acclimation time, this organism could transport and metabolize phenol. After 9 h of incubation with phenol as a secondary substrate, *Pseudomonas* sp. 9S8D2 had removed 44% of the phenol from the culture medium. The calculated removal rate in the presence of acetate as the primary substrate was 2.2 ± 0.2 nmol/mg of protein. These results suggest that *Pseudomonas* sp. 9S8D2 is able to grow when presented with acetate and phenol simultaneously.

To determine the capability of *Pseudomonas* sp. 9S8D2 to utilize phenol as the sole source of carbon and energy, cells were incubated in the presence of various concentrations of phenol in MSM, with phenol as the sole carbon source, for 16 h (Fig. 2). Growth was assessed by optical density and total cellular protein measurements during concomitant loss

of phenol from the culture supernatants. Phenol toxicity was noted for concentrations in excess of 225 μg/ml. At concentrations of 125 to 200 μg/ml, in which good growth was observed, the rate of phenol removal was constant and was approximately 22 nmol/mg of protein. This is a 10-fold increase over the removal rate measured for phenol in the presence of acetate at primary substrate concentrations. These results suggest that the rate of removal of a toxic organic compound, such as phenol, can be influenced by the presence of naturally occurring substrates.

In many bacterial genera (16, 19, 27), as well as *Candida* sp. (15, 22), enzymes of the phenol degradation pathway are inducible, their synthesis being elicited by the phenolic substrate or metabolic intermediates. Inducibility of phenol-degrading enzymes in strain 9S8D2 was tested by precultivating cells on 1mM acetate or 1 mM phenol, then supplying 1 mM phenol as the sole carbon and energy source for both groups. Those cells precultivated on phenol began degradation without a lag period and had taken up 98% of the phenol after 3 h of incubation. In contrast, those cells precultivated on acetate showed a 3- to 4-h lag period before the onset of phenol degradation and had taken up only 75% of the phenol after 12 h of incubation. Thus, as in other cell types tested, the enzymes of phenol degradation are inducible.

Both stimulatory and repressive effects of natural substrates on removal of xenobiotic compounds have been reported, but the former effects are usually the result of primary substrates. To investigate the role that acetate plays as either a primary or secondary substrate during phenol removal, *Pseudomonas* sp. 9S8D2 was exposed to 1 mM phenol alone (control) and with 0.1, 1, 5, and 10 mM acetate for 8 h (Fig. 3). Only when acetate was present at equimolar or lower concentrations was phenol utilization enhanced. In contrast, when acetate was present at primary substrate concentrations (5 and 10 mM), proportionately less phenol was removed. Similarly, studies with *Alcaligenes eutrophus*

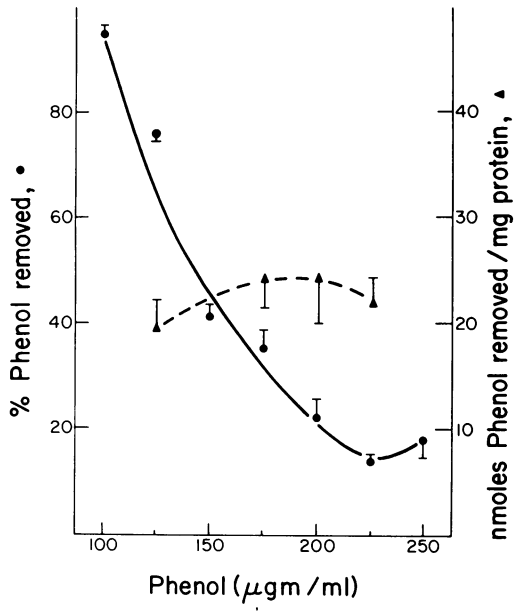

FIG. 2. Removal of phenol from MSM by strain 9S8D2. Sixteen-hour incubations were initiated by addition of resuspended cells to medium containing the indicated concentrations of phenol as sole carbon and energy source. Symbols represent the mean of duplicate cultures; bars indicate the range between duplicates.

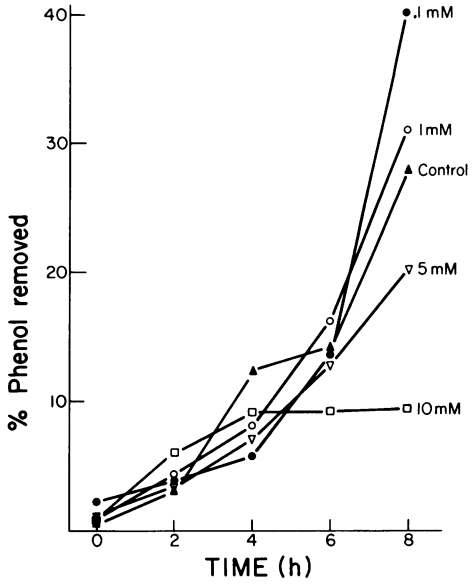

FIG. 3. Effect of acetate concentration on removal of phenol by *Pseudomonas* sp. 9S8D2. Dense cell suspensions were incubated with 100 μg of phenol per ml in MSM alone (control) and with 0.1, 1, 5, or 10 mM acetate. Symbols are the averages of duplicates which varied by less than 10%.

335 (16) cultivated with high concentrations of acetate (20 mM) and either phenol or *p*-cresol did not reveal detectable phenol hydroxylase activity. Taken together, these results indicate that relative substrate concentration is an important consideration in investigating natural compound-xenobiotic compound interactions.

Characterization of acetate metabolism. Measurement of microbial acetate metabolism is most commonly studied by using radioligands, though gas chromatography and enzymatic phosphorylation by acetokinase methods have been described (28, 29). Use of such methods requires cell fractionations, extractions, and product derivatization. In contrast, application of solid-state NMR spectroscopy to the study of microbial degradation allows direct examination of carbon in all of the sample components of the intact lyophilized cells.

The experiment depicted in Fig. 1 was repeated, and phenol removal was monitored while dilution of [1-^{13}C]NaOAc into major cellular functional groups was assessed at 24 h by NMR spectroscopy. At this time, no detectable supernatant phenol remained. The ^{13}C CP-MAS NMR spectra of lyophilized intact *Pseudomonas* sp. 9S8D2 in the presence of 5 mM [1-^{13}C]NaOAc with and without 1 mM phenol is shown in Fig. 4a and b, respectively. A spectrum of solid [1-^{13}C]NaOAc consists of a doublet at 180 and 181.5 ppm. No peak in the 180-to-182-ppm region of the spectra of Fig 4 suggests that the intracellular levels of free acetate are negligible. However, the signal of 174 ppm, the ester-linked carboxyl carbon region of the ^{13}C NMR spectrum, is probably due to labeled acetyl coenzyme A. Increased NMR sensitivity from growth on ^{13}C-enriched acetate is evident when the spectrum in Fig. 4b is compared with the spectrum in the same experiment conducted with natural abundance levels (1.1%) of acetate (Fig. 5). Thirty thousand accumulations were necessary to approach an acceptable signal/noise ratio when unlabeled acetate was used. Similarity between the natural abundance level and

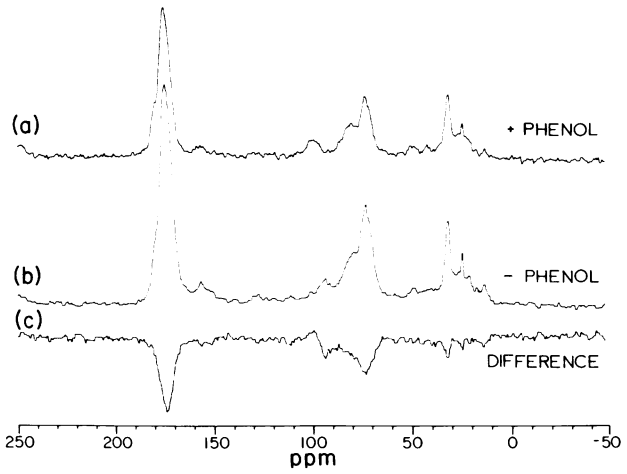

FIG. 4. Some 50.3-MHz ^{13}C CP-MAS NMR spectra of *Pseudomonas* sp. 9S8D2 grown for 24 h on 5 mM [1-^{13}C]NaOAc and on a natural abundance concentration (1 mM) of phenol. Spectra are of 25-mg weight-matched samples of lyophilized intact cells and represent 5,000 transients with phenol and ^{13}C-enriched acetate (a) and with ^{13}C-enriched acetate alone (b). The spectrum in (c) is the difference of (a) − (b). These spectra are typical of experiments performed at least three times. The chemical shift scale is in parts per million measured downfield from solid tetramethylsilane. Spinning sidebands are visible at approximately 100 ppm.

enriched acetate (Fig. 4b) spectra suggests that ^{13}C enrichment at the carboxyl carbon of acetate did not alter its metabolism by the pseudomonad. The difference spectrum in Fig. 4c was produced by the subtration of the spectra of samples grown without 1 mM phenol (Fig. 4b) from those grown with 1 mM phenol (Fig. 4a) and matched for weight and NMR parameters.

It is evident from enrichment of the main functional groups that extensive acetate metabolism took place. Dilution of the acetate label in such studies may be explained by fluxes through the TCA and glyoxylate cycles (10, 11). When [1-^{13}C]NaOAc is fed to microorganisms, the ^{13}C label appears in the carboxyl carbons of TCA and glyoxylate cycle intermediates. This partially accounts for the intense peak in the 170-to-180-ppm region of the spectrum, a region also including the carboxyl groups involved in ester, as well as peptide, linkages. From work with authentic compounds, as well as from the reports of others (17, 18, 32), the other main functional groups occur in the 60-to-110-ppm region, which includes hydroxymethyl and hydroxymethylene carbons of polysaccharides; the 11-to-15-ppm region, which contains terminal methyl carbons of fatty acids; the 24-to-30-ppm region, which includes the methylene carbons of fatty acyl chains; and an aromatic carbon region at 135 to 160 ppm. Similarities of the scaled spectra with and without 1 mM phenol present as a secondary substrate corroborate the phenol data (Fig. 1, 2, and 3) by suggesting that acetate is the preferred substrate. The lower peak intensities in the presence of phenol do not reflect a redistribution of acetate label within the sample but rather a difference in the amount of label per unit cell mass. A weight-matched amount of acetate- and phenol-grown cells was compared with the total yield of cells grown with acetate alone. This difference is not unexpected and has been reported for freshwater microorganisms in which 1 μg of phenol per ml was sufficient to increase growth rate above that obtained with indigenous nutrients (31). Data not shown indicated that, at 8 h of incubation with 5 mM labeled acetate with or without 1 mM phenol, there were no significant spectral differences. This suggests that, during rapid cell growth, the presence of phenol as a secondary substrate is not repressing the glyoxylate pathway. When microorganisms are grown on two-carbon compounds, such as acetate, two oxidative reactions linked to electron transport are bypassed by the glyoxylate pathway. Thus, rather than losing carbon as CO_2, and two two-carbon units are assimilated and appear in the major functional group regions of the solid-state NMR spectrum.

In a manner similar to the experiment described in the legend to Fig. 3, cells were grown for 24 h with 0.1 mM [1-^{13}C]NaOAc and 1 mM phenol. Phenol now serves as the primary substrate, whereas the naturally occurring substrate, acetate, is the secondary carbon source. Scaled ^{13}C CP-MAS NMR spectra of intact lyophilized cells appear in Fig. 6. A redistribution of the acetate label is clearly evident when phenol is present; this is reflected in the difference spectrum (Fig. 6c). In the presence of phenol as the primary substrate, relative intensities in the methyl carbon and acyl chain methylene carbon regions are increased two- to fourfold over the peak intensities of acetate alone. Another peak is emerging in this spectrum at 55 to 62 ppm, a range attributable to methylene carbons from amino acids (17). In contrast, the intensity of the peaks in the carbohydrate region (60 to 90 ppm) is decreased twofold in the presence of phenol, as evidenced by the negative intensity at ca. 73 ppm in the difference spectrum (Fig. 6c). These results indicate that, in the presence of phenol at higher concentrations, a redistribution of acetate occurs, and that it is preferentially shuttled into fatty acyl chain synthesis, probably via acetyl

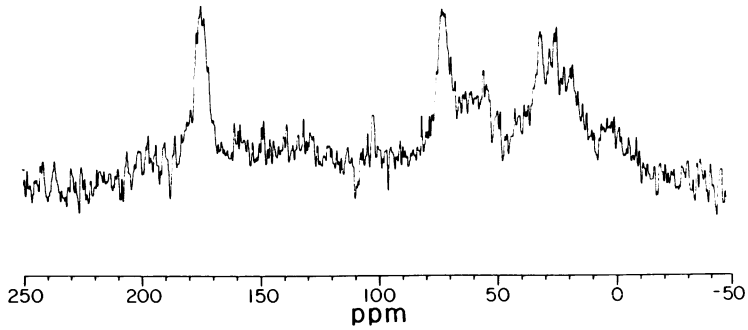

FIG. 5. A 50.3-MHz ^{13}C CP-MAS NMR spectrum (30,000 accumulations) of cells of strain 9S8D2 grown on a natural abundance concentration of acetate (5 mM).

coenzyme A. The decreased intensity of the acetate label in the carbohydrate region of the +phenol spectrum suggests that phenol carbon is being funneled into the TCA cycle.

Taken together, these results show that simultaneous use of a xenobiotic compound and a natural substrate does occur and that primary and secondary substrate concentrations do influence the rate and manner in which the compounds are utilized.

DISCUSSION

We isolated a sediment-dwelling *Pseudomonas* sp. from an environment in which groundwater is free from organic pollutants and demonstrated that it is capable of metabolizing phenol as a sole carbon source or in the presence of the naturally encountered substrate acetate. Similar results have been reported for *Pseudomonas acidovorans*, which simultaneously grew on uncharacterized dissolved organic carbon while mineralizing phenol present at 2 μg/liter (34). Similarly, metabolism of 3-methyl-4(methylthio)phenol was influenced by the presence of acetate and sucrose as carbon sources (26). With acetate, high 2,3-dioxygenase activity led to complete xenobiotic compound degradation, whereas sucrose induced a higher level of 1,2-dioxygenase activity, leading to the formation of two recalcitrant metabolites. In these studies, the degradative products varied with the type of cosubstrate.

Enzymes responsible for degradation of phenol and related compounds have been shown to be induced by either the xenobiotic compound or a metabolic intermediate (15, 16, 19, 22, 27). Our data suggest that these enzymes are also induced in *Pseudomonas* sp. 9S8D2.

Stimulatory and repressive effects of natural substrates on the removal of xenobiotic compounds have been reported. In a general type of investigation, a lake microbial community was adapted to increasing concentrations of amino acids, carbohydrates, or fatty acids, then presented with *m*-cresol, *m*-aminophenol, or *p*-chlorophenol. Enhanced degradation was observed, suggesting that prior exposure to labile, natural substrates can significantly enhance the ability of a microbial community to respond to xenobiotic compounds (36).

In a series of pure-culture experiments, *P. acidovorans* began mineralizing phenol 16 to 22 h after incubation with acetate in 50- to 200-fold excess over phenol (34). However, when acetate and phenol were present at 13 and 2 μg/liter, respectively, simultaneous mineralization was noted. Taken together, the phenol and [1-^{13}C]NaOAc data of the present investigation indicate a delay in the onset of phenol metabolism when a natural substrate is present in excess. There is also evidence for simultaneous metabolism, particularly at lower concentrations, at which the presence of acetate at secondary substrate concentrations enhanced phenol metabolism. This is in accord with the recent report on *Pseudomonas* sp. LP, for which acetate concentrations up to 1 mg/liter were stimulatory for removal of 100 μg of methylene chloride per ml, even though *Pseudomonas* sp. LP showed a preference toward degrading methylene chloride, whether it was the primary or secondary substrate (23). The pseudomonad in our investigations showed a phenol removal rate of 2 nmol/mg of protein in the presence of primary substrate concentrations of acetate. This rate was increased 10-fold when phenol was supplied as the sole carbon source.

In a novel approach to the study of substrate interactions during xenobiotic compound degradation, we have adapted a natural-abundance-level ^{13}C CP-MAS NMR spectroscopic technique commonly used for the study of solid fossil fuels and their diagenetic precursors (8, 9). Use of ^{13}C-enriched substrates increases the sensitivity of this technique enough to allow monitoring of a label introduced into a sample in the micromolar range. In our investigations, we have used this method to demonstrate the dilution and redistribution of a ^{13}C-enriched natural substrate in the presence of phenol as the xenobiotic compound. A redistribution of the acetate label into the acyl fatty acid chain and methyl carbon region of the NMR spectra and a dilution of label in the carbohydrate region were evident when acetate was present in secondary substrate concentrations. These redistributions indicate that phenol may be incorporated into the polysaccharides, but not the lipids, of strain 9S8D2. Results of recent studies of a freshwater microbial consortia enriched with the phenol-utilizing population demonstrate mineralization of 80% and incorporation of 20% of the phenol present at 1 μg/ml (7). Phenol carbon was found in the TCA-precipitable fraction, suggesting that it was incorporated into cellular nucleic acids, proteins, and polysaccharides but not the lipid fraction. Our data support these results. This suggests that the handling and incorporation of carbon can vary with changes in concentrations of indigenous nutrients. We are currently conducting experiments with ^{13}C-enriched pollutant molecules to understand these phenomena more fully.

To our knowledge, only one other microbial degradation study using ^{13}C CP-MAS NMR spectroscopy has been published to date. In a series of very elegant experiments,

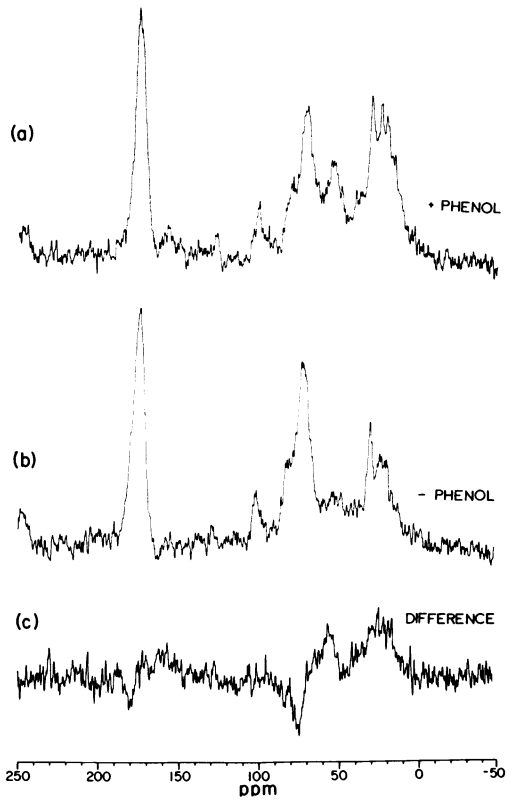

FIG. 6. Some 50.3-MHz ^{13}C CP-MAS NMR spectra of *Pseudomonas* sp. 9S8D2 grown for 24 h on 0.1 mM [1-^{13}C]NaOAc and 1 mM (natural abundance concentration) phenol. Spectra are of 70-mg weight-matched samples, analyzed under the same conditions as described in the legend to Fig. 4.

the complete degradative pathway of the broad-spectrum herbicide glyphosate by a soil pseudomonad was identified (17). The unique advantages of the solid-state NMR spectroscopic technique include direct examination of the entire lyophilized sample. There is no loss of material due to harsh fractionations or extractions. Though our investigation, as well as the glyphosate study, was conducted with pure bacterial cultures in liquid media, application of the technique to pure cultures or microbial consortia in intact sediment samples is possible, as we have demonstrated in our geochemical work (8, 9).

The importance of establishing microbial transformations in natural ecosystems such as sewage, sediments, soils, and estuaries to assess the effects of complexing materials and mineral surfaces and the impact of competing species on the operant degradative pathways has been stressed (1). Recent work on the effects of chemical concentration, indigenous microbial population, and ecosystem characteristics on degradation of *tert*-butylphenyl diphenyl phosphate have been published (14). Conventional methods were used to show that microbial degradation took place via three processes and was most extensive when low concentrations of the xenobiotic compound were introduced to sediment microorganisms in a eutrophic environment previously exposed to anthropogenic chemicals. Solid-state NMR spectroscopic methods could be easily adapted to such projects.

The principle advantage of solid-state NMR spectroscopy in microbial degradation studies is that it is both nondestructive and noninvasive. Furthermore, the inherent insensitivity of NMR can be overcome by the use of labeled substrates. The use of site-labeled compounds (e.g., [1-^{13}C]NaOAc) also allows the distribution of specific functional groups in substrate molecules to be observed directly. Although it has not been demonstrated that solid-state NMR is quantitatively reliable (12, 13), the method is sufficiently semiquantitative to discern two- to fourfold increases in functional group peak intensities (Fig. 6). Optimization studies and careful control of pulse sequences can further enhance the reliability of the technique, as we have previously demonstrated in our geochemical studies (8, 9). Ultimately, however, it is the direct comparison of relative intracellular concentrations of specific carbon functional groups afforded by the solid-state NMR method, not the absolute intracellular concentrations, that is relevant in studies such as this. We anticipate that the solid-state technique will become a tool that will complement conventional spectroscopic and radiolabel techniques, as well as high-resolution NMR methods (4, 5), as these instruments become more accessible to microbiologists.

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